

## What Do Mexican Fruit Flies Learn When They Experience Fruit?

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*Mexican fruit flies learn fruit characteristics that enable them to distinguish familiar fruits from novel fruits. We investigated whether mature Mexican fruit flies learn fruit color, size or odor. We found no evidence that female flies learn fruit color or size after experience with host fruit, including oviposition. However, green fruit and fruit models were more attractive than yellow and red fruit and fruit models regardless of previous experience. Females with grapefruit experience were more attracted to fruit models with extract of either grapefruit peel or pulp, than to models without extract. Females with no experience with grapefruit were not attracted to models treated with grapefruit extract. These results indicate that females learned fruit odor during exposure to grapefruit.*

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**KEY WORDS:** *Anastrepha ludens*; fruit fly; host; attraction; oviposition; experience; learning; odor.

### INTRODUCTION

Numerous studies have investigated the effects of learning on host finding behavior in several species of tephritid fruit flies including *Rhagoletis pomonella* (Walsh), *Ceratitis capitata* (Wiedemann), *Bactrocera tryoni* (Froggatt), *B. dorsalis* (Hendel), and others (Prokopy *et al.*, 1990; Fletcher and Prokopy, 1991). These studies generally demonstrated that experience

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with host fruit modifies foraging for and acceptance of both the experienced fruit and other fruit by fruit flies. However, relatively few studies have addressed the question of what flies learn when they experience fruit.

The Mexican fruit fly, *Anastrepha ludens* (Loew), is highly polyphagous with over 30 reported field hosts and many additional hosts that are suitable in the laboratory (Norrbom and Kim, 1988). It is a serious pest of both citrus and mangoes in Mexico and Central America, and threatens the citrus industry in the United States. Despite much research on this insect, remarkably little is known of its host finding behavior.

One aspect of the host finding behavior of the Mexican fruit fly that has been studied recently is the role of learning. In wind-tunnel experiments with various fruits, both wild and laboratory-strain females showed little or no innate response to fruits, but responses by both fly types increased after experience with fruit. Also, both fly types showed cross induction (Jaenike, 1983) in which exposure to one fruit type increased attraction to similar fruit types. These results were observed in experiments with grapefruits only (Robacker and Fraser, 2002a), grapefruits vs. chapote (a native host) fruits (Robacker and Fraser, 2002b), and grapefruits vs. oranges (Robacker and Fraser, 2003). In these experiments, responses to the experienced fruit increased more than responses to similar fruit that the flies had not experienced indicating that flies learned to distinguish fruit from each other after experience with only one of the fruit types, a surprising result in the case of grapefruits and oranges that were similar in color (yellow-orange vs. orange), size (ca. 9–10 cm vs. ca. 6–7 cm diam), and aromas. These results indicate that the flies learned one or more characteristics of the fruit they experienced that allowed them to distinguish fruits from each other.

The present work was undertaken to discover what types of cues Mexican fruit flies learn about fruit they experience. Several experiments were conducted to evaluate learning of fruit color, fruit size, and fruit aroma using wind-tunnel bioassays like those used in the experiments described above. Fruit color was evaluated using red, yellow and green bell peppers or fruit models after exposure to red, yellow, or green bell peppers. Fruit size was evaluated using chapote fruit size vs. grapefruit size models after experience with chapote or grapefruit, and orange size vs. grapefruit size models after experience with oranges or grapefruits. Interaction of color and size was evaluated using small orange-colored models vs. large orange-colored models after experience with small green chapote fruits or large yellow-orange grapefruits. Finally, fruit odor was evaluated using models containing extracts of grapefruit peel or pulp vs. no odor after experience with grapefruits. Behavior of both males and females was measured because both sexes are known to occur on fruit for feeding (Aluja *et al.*, 1989), mating (forced matings by males) (Robacker *et al.*, 1991), and/or oviposition.

Although behavior of laboratory-strain and wild flies was qualitatively similar in the wind-tunnel experiments discussed above (Robacker and Fraser, 2002a; 2002b; 2003), laboratory flies were used in these experiments because they typically respond at much high rates than wild flies in wind-tunnel assays.

## MATERIALS AND METHODS

### Insects and Handling Methods

Mexican fruit flies (*A. ludens*) used in this study originated from pupae collected in 1997 from yellow chapote fruit (*Casimiroa greggii* S. Wats.) (Rutaceae), a native host from northeastern Mexico, and have been in laboratory culture for more than 50 generations. Flies are reared on artificial medium after egg collection in red-colored, xanthan-gum gel. Adults used in this work were held in Plexiglas cages ( $20.5 \times 20.5 \times 20.5$  cm) with screened tops containing a diet mixture of sugar and yeast hydrolysate, with water supplied separately. In addition, most cages were provisioned with one of several types of fruits beginning 1–2 days after eclosion, as will be described for each experiment (below). Flies were used in experiments when 14–28 days old. This age range was based on observations of oviposition behavior in previous work (Robacker and Fraser, 2002b). Laboratory conditions where flies were housed were  $22 \pm 2^\circ\text{C}$  and  $50 \pm 20\%$  relative humidity with a photophase of 0630 to 1930 h provided by fluorescent lights. Experiments were conducted between 0900 and 1700 h. Foraging for fruit was relatively uniform during this time span in previous experiments (Robacker and Fraser, 2001).

### Wind-Tunnel Bioassay

Bioassays were conducted in a Plexiglas wind tunnel ( $0.3 \times 0.3 \times 1.2$  m) screened on each end to allow airflow. The downwind end contained a baffle system to create uniform airflow through the chamber. Air was pulled through the chamber at 0.4 m/sec by an exhaust fan connected to the downwind end. The top of the chamber had two circular openings (12.8 cm diameter) with Plexiglas covers, one located at each end of the chamber, to allow easy access to the chamber's interior. A 75 W "soft white" light bulb (General Electric Co., Cleveland, OH) in a reflecting lamp was positioned 17 cm above the downwind end of the chamber. The purpose of this light was to use the flies' positive phototactic reaction to minimize random flying into the upwind end of the chamber that could result in accidental landings on

fruit models. Overhead lighting was provided by fluorescent “cool white” lights (F40CW, General Electric).

About 24 flies (11–12 of each sex) were transferred into pint-size, cylindrical paper cups with screen tops 24 h prior to testing. Flies were held in cups without food or fruit but water was provided. Previous work indicated that oviposition-ready flies responded to grapefruits at higher rates after 1–2 days of sugar deprivation (Robacker and Fraser, 2001). To conduct trials in all experiments except Experiment II (described below), one or more fruit models were placed on a chicken-wire platform suspended from the opening in the upwind end of the chamber, and one cup of flies was placed under the downwind opening. Flies were allowed 5 min to leave the cup and respond to the model. Upwind movement was scored if flies passed a point  $2/3$  of the distance from the release cup to the model. Landing was scored for either landing or walking onto the model. Each trial of all experiments was conducted with a different cup of flies.

### **Experiment I: Fruit Color Using Models in No-Choice Test**

This experiment was to test if flies learned fruit color. Holding cages were provisioned with green, yellow or red bell peppers (*Capsicum annuum* L.) (Solanaceae), or were not provisioned with fruit. Bell peppers are readily accepted as hosts in laboratory tests but have not been reported as field hosts of the Mexican fruit fly (Norrbon and Kim, 1988). Twelve fruit-model  $\times$  color-experience treatments (color of fruit in holding cage), per replication, were tested in random order: three model-colors (green, yellow, red)  $\times$  four color-experience treatments (green peppers, yellow peppers, red peppers, no fruit). Model colors were prepared after scanning bell peppers with a Model CFS57U Spectrophotometer (X-Rite, Inc., Grandville, Michigan) with Match Rit software (version 1.20.0) (X-Rite) and mixing paints (Ace Royal Accent, 100% acrylic latex, flat accent colors, Ace Hardware Corp., Oak Brook, Illinois) to match the fruit colors. Styrofoam balls purchased at a local hobby store (9.7 cm diam.) painted with these colors were used as models. Thirteen replications were conducted.

### **Experiment II: Fruit Color Using Fruit in Choice Test**

This experiment was similar to the previous one except that bell peppers were used instead of models and all three colors of bell peppers were tested simultaneously on the bottom of the upwind end of the wind tunnel. The same four color-experience fly treatments as in the previous

experiment were tested, one per trial. The positions of the colored fruits were the same for all four trials within a replication of the four color-experience groups, but were varied for each replication. The same three fruits were used for all four trials of a replication. Fruits were washed with water between trials. Nineteen replications were conducted.

### Experiment III: Fruit Size

This experiment was to test if flies learned fruit size. Holding cages were provisioned with either an orange-colored Valencia orange (*C. sinensis* (L.) Osbeck) or a yellow-orange-colored Rio Red grapefruit (*C. paradisi* MacFadyen), or were not provisioned with fruit. Both types of fruit are readily infested by Mexican fruit flies in the field (Norrbon and Kim, 1988). Oranges averaged 6–7 cm and grapefruits 9–10 cm diam. Although these sizes are not greatly different, Mexican fruit flies were able to distinguish grapefruits and oranges in previous work (Robacker and Fraser, 2003) indicating flies learned some cue(s) that differed between the two fruit types. Six fruit-model  $\times$  size-experience treatments, per replication, were tested in random order: two model sizes (9.7 cm and 6.2 cm diam. orange balls)  $\times$  three size-experience treatments (grapefruit, orange, no fruit). The orange paint (Ace Hardware) used to prepare styrofoam fruit models approximated a color intermediate between the orange color of oranges and the yellow-orange color of the grapefruits, as judged by human vision. Sixteen replications were conducted.

### Experiment IV: Fruit Size $\times$ Color

This experiment was conducted to re-assess size using two greatly different fruit sizes, and to test learning of fruit size in combination with color. Holding cages were provisioned with either green-colored “yellow-chapote” fruits or yellow-orange-colored grapefruits, or were not provisioned with fruit. Yellow chapote is a native host of this fly (Norrbon and Kim, 1988). Chapote fruits were 1–2 cm diam. and grapefruits 9–10 cm diam. Six fruit-model  $\times$  size/color-experience treatments, per replication, were tested in random order: two model sizes (2.2 cm and 9.7 cm diam. orange balls)  $\times$  three size/color-experience treatments (chapote, grapefruit, no fruit). For trials with the small models, six balls were placed on the screen platform to reproduce the appearance of the groups of chapote fruits in the holding cages. Models were styrofoam balls painted with the same orange paint used in Experiment III. Twenty-two replications were conducted.

### Experiment V: Fruit Volatiles

This experiment was conducted to determine if Mexican fruit flies learn the odor of fruit. Holding cages were provisioned with Rio Red grapefruits with a portion (2 cm diameter) of the peel removed to expose the pulp, or no fruit. Extracts of both peel and pulp were prepared for use as odor sources in the bioassays. Peel extracts were made to mimic the smell of grapefruit essential oils and pulp extracts were made because experiments have shown that pulp wounds in fruit increase attractiveness and/or acceptance to fruit flies (Papaj *et al.*, 1989; Prokopy *et al.*, 1990; Katsoyannos *et al.*, 1997; Robacker and Fraser, 2002a). Grapefruit peel and pulp were extracted with high purity methylene chloride (Burdick and Jackson, Baxter Healthcare Corp., Muskegon, MI) and high purity acetone (Burdick and Jackson), respectively. Extracts were made by cutting and weighing about 10 g of whole peel (albedo and flavedo) and about 10 g of pulp and putting each into a vial containing 50 ml of the respective solvents. The vials were agitated for 24 h, then the extracts were filtered through 0.45 mm nylon luer-lock syringe filters (Alltech Associates, Inc., Deerfield, IL). Methylene chloride filtrates were concentrated under nitrogen to 5 ml and acetone extracts to 10 ml.

Peel and pulp extracts were tested in separate experiments. For each experiment, four fruit-odor  $\times$  fruit-experience treatments, per replication, were tested in random order: two fruit-odor models (ball with fruit extract, ball with solvent)  $\times$  two fruit-experience treatments (grapefruit, no fruit). Fruit-odor models were styrofoam balls (diam. 9.7 cm) painted with the same orange paint used in Experiment III. Odor treatments consisted of 100  $\mu$ l of peel extract, pulp extract, or solvent, on a piece of filter paper fastened to the top of the fruit model. Fruit models were not placed into the wind tunnel until solvent had evaporated. Eighteen replications were conducted using peel extracts, and 16 using pulp extracts.

### Statistical Analyses

The proportions of flies that responded to fruits or models were transformed by arcsin of the square root for data analyses (Snedecor and Cochran, 1967). Proportions of males or females that moved upwind, or landed on the fruit or ball, were calculated as the number of males or females that performed the behavior divided by the number that was available to perform the behavior (*a*). For Experiments I, III, and IV, "*a*" was the number that left the cup; for Experiments II and V, "*a*" was the total number (of males or females) in the cup. The rationale for using different

denominators was that all flies (both in and out of the cups) would be able to perceive volatiles from fruit (Experiment II) or models with applied volatiles (Experiment V), but only flies that actually left cups could perceive colors or sizes of models (other experiments). Proportions of 0 were replaced with  $1/4a$  before transformation (Snedecor and Cochran, 1967).

Results (transformed proportions) of Experiments I, III, IV and V were analyzed by factorial analysis of variance with replication (SuperANOVA, Abacus Concepts, 1989). Additional non-factorial analyses were performed to determine the overall treatment effect (for all test model (or fruit) by experience treatments). Means separations were conducted using Fisher's protected least significant difference method (Snedecor and Cochran, 1967). Results (transformed proportions) of Experiment II were analyzed by split-plot analysis of variance in which experience treatments were main plots and test fruits were split plots (SuperANOVA, Abacus Concepts, 1989). Separate analyses were conducted for males and females in all experiments.

## RESULTS

### Experiment I: Fruit Color Using Models in No-Choice Test

Responses of females to green, yellow and red fruit models following experience with green, yellow or red bell peppers, or having had no previous experience with fruit, are shown in Table I. Significant differences among means related entirely to model color. More females moved upwind toward green than red models ( $P < 0.05$ ,  $F = 4.0$ ,  $df = 2,132$ ) and landed on green than on either yellow or red models ( $P < 0.05$ ,  $F = 4.5$ ,  $df = 2,132$ ). Experience had no effect on attraction to models. The model color X experience interaction was significant for upwind movements ( $P < 0.05$ ,  $F = 2.4$ ,  $df = 6,132$ ), however, no biologically meaningful interpretation was evident. No significant effects were found in analyses of male behavior.

### Experiment II: Fruit Color Using Fruit in Choice Test

Responses of females given a choice of green, yellow and red bell peppers following experience with green, yellow or red bell peppers, or having had no previous experience with fruit, are shown in Table II. More females landed on green or red than on yellow fruit ( $P < 0.001$ ,  $F = 7.5$ ,  $df = 2,144$ ). Responses to green were not significantly higher than to red

**Table I.** Percentages of Female Mexican Fruit Flies With No Fruit Experience, or With Experience with Green, Yellow, or Red Bell Peppers, Attracted to Green, Yellow, or Red Fruit Models in a Wind Tunnel<sup>a</sup>

Fruit-color experience	Model color	Moved upwind	Landed on model
None	Green	19.2 ± 3.5 bcd	9.0 ± 2.9 c
None	Yellow	13.9 ± 3.5 abc	1.1 ± 1.1 a
None	Red	14.9 ± 4.9 abc	2.5 ± 1.7 ab
Green	Green	11.8 ± 3.3 abc	3.5 ± 1.8 ab
Green	Yellow	20.6 ± 4.1 cd	4.5 ± 2.0 abc
Green	Red	8.1 ± 2.8 a	3.2 ± 2.3 ab
Yellow	Green	25.6 ± 6.5 d	7.7 ± 2.7 bc
Yellow	Yellow	13.3 ± 3.4 abc	3.5 ± 2.0 ab
Yellow	Red	10.7 ± 4.7 ab	2.0 ± 1.3 a
Red	Green	20.6 ± 4.4 cd	4.4 ± 2.6 abc
Red	Yellow	10.6 ± 2.9 ab	3.2 ± 1.7 ab
Red	Red	16.2 ± 3.9 abcd	1.6 ± 1.1 a

<sup>a</sup>Values are mean percentages (±SE) of females responding out of the total females that left the holding cup (mean females (±SE) per trial = 10.4 ± 0.1; *n* = 13 trials per experience/model-size treatment). Means followed by different letters in the same column are significantly different at the 5% level by Fisher's protected LSD.

fruit. Experience had no effect on either upwind movement or landings on the test fruit. This was indicated both by nonsignificant main effects of experience, and by nonsignificant interaction (analyzed only for landings) of experience-fruit color with test-fruit color. No significant effects were found for males.

### Experiment III. Fruit Size

Responses of females to orange-sized vs. grapefruit-sized fruit models following experience with oranges or grapefruits, or having had no previous experience with fruit, are shown in Table III. Large and small fruit models did not differ in attractiveness to females regardless of their previous experience. Behavior of males also was not affected.

### Experiment IV: Fruit Size X Color

Responses of females to chapote-sized vs. grapefruit-sized, orange-colored fruit models following experience with chapote fruits, grapefruits, or no fruits, are shown in Table IV. The two fruit models did not differ in attractiveness to females regardless of their previous experience. Behavior of males also was not affected.



**Table II.** Percentages of Female Mexican Fruit Flies With No Fruit Experience, or With Experience with Green, Yellow, or Red Bell Peppers, that Landed on Green, Yellow, or Red Bell Peppers in a Choice Test in a Wind Tunnel<sup>a</sup>

Fruit-color experience	Moved upwind	Test-fruit color	Landed on fruit
None	12.9 ± 3.2	Green	1.4 ± 1.0
		Yellow	0.0 ± 0.0
		Red	2.1 ± 1.2
Green	21.4 ± 4.0	Green	5.5 ± 2.1
		Yellow	1.9 ± 1.2
		Red	1.4 ± 0.8
Yellow	16.9 ± 2.2	Green	5.3 ± 1.4
		Yellow	0.5 ± 0.5
		Red	3.2 ± 1.4
Red	17.2 ± 2.8	Green	2.6 ± 1.0
		Yellow	0.4 ± 0.4
		Red	2.9 ± 1.0

<sup>a</sup>Values are mean percentages (±SE) of females responding out of the total females in the holding cup (mean females (±SE) per trial = 10.1 ± 0.1; *n* = 19 trials per experience treatment). No “moved upwind” means were significantly different at the 5% level by Fisher’s protected LSD. Means separations could not be performed on the individual means for the split plot variable “landed on fruit.”

Experiment V. Fruit Volatiles

Responses of females to fruit models with grapefruit extracts applied to them are shown in Table V. Models with peel extract were more attractive than models with methylene chloride to grapefruit-experienced females but were not more attractive than those with solvent to naïve (inexperienced) females (upwind movements: *P* < 0.001, *F* = 6.2, *df* = 3,68; landings:

**Table III.** Percentages of Female Mexican Fruit Flies With No Fruit Experience, or With Experience with Grapefruit or Oranges, Attracted to Grapefruit-Sized or Orange-Sized Fruit Models in a Wind Tunnel<sup>a</sup>

Fruit experience	Model size	Moved upwind	Landed on model
None	Grapefruit	12.9 ± 2.2	2.3 ± 1.0
None	Orange	7.1 ± 1.9	1.6 ± 0.8
Grapefruit	Grapefruit	10.6 ± 4.3	1.1 ± 0.7
Grapefruit	Orange	14.5 ± 2.9	1.7 ± 1.2
Orange	Grapefruit	13.2 ± 2.9	2.5 ± 1.2
Orange	Orange	12.5 ± 2.4	0.0 ± 0.0

<sup>a</sup>Values are mean percentages (±SE) of females responding out of the total females that left the holding cup (mean females (±SE) per trial = 9.9 ± 0.2; *n* = 16 trials per experience/model-size treatment). No means in the same column were significantly different at the 5% level by Fisher’s protected LSD.

**Table IV.** Percentages of Female Mexican Fruit Flies With No Fruit Experience, or With Experience with Grapefruit or Chapote Fruit, Attracted to Grapefruit-Sized or Chapote-Sized, Orange-Colored Fruit Models in a Wind Tunnel<sup>a</sup>

Fruit experience	Experienced color	Model size	Moved upwind	Landed on model
None	None	Grapefruit	11.4 ± 2.3	1.6 ± 1.0
None	None	Chapote	14.3 ± 2.5	2.7 ± 1.0
Grapefruit	Yellow/orange	Grapefruit	17.2 ± 2.8	3.0 ± 1.3
Grapefruit	Yellow/orange	Chapote	15.7 ± 2.3	3.1 ± 1.1
Chapote	Yellow/green	Grapefruit	15.7 ± 2.3	1.6 ± 0.9
Chapote	Yellow/green	Chapote	9.9 ± 1.7	1.7 ± 0.9

<sup>a</sup>Values are mean percentages (±SE) of females responding out of the total females that left the holding cup (mean females (±SE) per trial = 10.1 ± 0.2; *n* = 22 trials per experience/model-size treatment). No means in the same column were significantly different at the 5% level by Fisher's protected LSD.

$P < 0.05$ ,  $F = 3.8$ ,  $df = 3,68$ ). Models with pulp extract were more attractive than models with acetone to grapefruit-experienced females but were not more attractive than those with solvent to naïve females (upwind movements:  $P < 0.05$ ,  $F = 3.6$ ,  $df = 3,45$ ; landings:  $P < 0.01$ ,  $F = 6.0$ ,  $df = 3,45$ ).

Responses to fruit extracts by males with or without experience with grapefruit were not statistically significant.

## DISCUSSION

Fletcher and Prokopy (1991) concluded that vision plays a dominant role in host fruit finding by fruit flies after arrival to host trees. Further,

**Table V.** Percentages of Female Mexican Fruit Flies, Naïve or With Previous Experience with Grapefruit, Attracted to Grapefruit Extracts in a Wind Tunnel<sup>a</sup>

Experiment	Fruit experience	Chemicals tested on model	Moved upwind	Landed on model
Peel	None	Methylene chloride	12.8 ± 2.5 a	0.5 ± 0.5 a
Peel	None	Peel extract	15.1 ± 3.0 a	0.5 ± 0.5 a
Peel	Grapefruit	Methylene chloride	20.4 ± 2.4 a	1.4 ± 0.8 a
Peel	Grapefruit	Peel extract	28.5 ± 3.3 b	4.4 ± 1.7 b
Pulp	None	Acetone	17.6 ± 3.6 a	1.0 ± 0.7 a
Pulp	None	Pulp extract	20.7 ± 3.2 a	3.7 ± 1.4 ab
Pulp	Grapefruit	Acetone	19.5 ± 3.3 a	0.0 ± 0.0 a
Pulp	Grapefruit	Pulp extract	33.1 ± 5.6 b	7.3 ± 2.1 b

<sup>a</sup>Values are mean percentages (±SE) of females responding out of the total females in the trial (mean females (±SE) per trial = 11.3 ± 0.08; *n* = 18 trials per treatment in the peel extract experiment and 16 trials per treatment in the pulp extract experiment). Means from the same experiment in the same column followed by different letters are significantly different at the 5% level by Fisher's protected LSD.

they stated that flies may recognize fruit by its color, shape, size and contrast against a background of foliage or the sky. However, despite much research indicating the importance of these fruit attributes, only a few experiments have addressed the question of whether flies learn them when they are exposed to fruit.

In the work we present here, mature female Mexican fruit flies exposed to various host fruits showed no increase in attraction to fruit models of colors or sizes similar to those of the experienced fruit. This was true for exposure to grapefruits, oranges, bell peppers, and yellow chapote, a native host, followed by testing with models differing in color by as much as red, yellow and green, and in size by as much as grapefruits (9.7 cm diam.) and yellow chapote fruits (2.2 cm diam.). Possible imperfect color matching of our model colors to the fruits could explain the negative results in Experiments I and III but not Experiment II in which flies were exposed to bell peppers then tested for color preference with bell peppers. Our negative results do not eliminate the possibility of subtle learning of fruit color and size that was not detectable with our bioassay. Also, we did not test the possibility that flies may only learn color and size in combination with odor.

Several studies have shown that female fruit flies learn fruit size but none indicated that they learned fruit color. Papaj and Prokopy (1986) showed that *R. pomonella* females learned hawthorn size after experience with hawthorn as indicated by subsequent rejection of apple-sized models. Papaj *et al.* (1989) showed that *C. capitata* females trained with large fruit accepted large fruit over smaller ones and those trained with small fruit accepted small fruit over larger ones. Likewise, Prokopy *et al.* (1989), working with mock orange and sweet orange fruit and fruit models, found that female *C. capitata* learned the size of fruit they experienced. In their study, Prokopy *et al.* (1989) found no evidence the flies learned fruit color. Finally, Prokopy *et al.* (1990) found that *B. dorsalis* females exposed to apples or kumquats, then given a choice of models of various sizes and colors, preferred models of the size of experienced fruit. Again, color had little effect. In fact, the only study that may have shown learning of color was that of Henneman and Papaj (1999) in which male *Rhagoletis juglandis* (Cresson) developed a preference for green models after experience with green models in the presence of females. Female *R. juglandis* always preferred green, regardless of prior experience. Thus, these studies agree with our results regarding lack of color learning, but disagree with our finding that size was not learned by female Mexican fruit flies.

Fletcher and Prokopy (1991) cited evidence that female fruit flies use host odors along with general visual cues to locate host trees but that host fruit odors have little effect on finding of fruit after flies arrive to host trees. As was the case with visual cues, only a few experiments have investigated

whether flies learn olfactory cues when they experience fruit and subsequently use them to locate host trees or fruits.

Our work demonstrated that female Mexican fruit flies that are exposed to grapefruits (with exposed peel and pulp) develop an attraction response to both peel and pulp chemicals. Based on the definition by Thorpe (1963) that learning is an adaptive change in behavior as a result of experience, our data indicate flies learned the odor of the fruit and used the information to search for grapefruits. While other explanations of the data are possible, researchers studying changes in host preferences in fruit flies as a result of fruit experience have not given serious consideration to non-learning mechanisms.

Studies with other fruit flies have also demonstrated or implicated learning of host fruit chemicals. Papaj and Prokopy (1986) demonstrated that females of *R. pomonella* exposed to hawthorn fruit accepted hawthorn-sized models that were impregnated with apple chemicals at lower rates than either naïve or apple-trained flies, thus indicating they had learned hawthorn chemicals and used the information to reject novel fruit-chemical stimuli. Also working with *R. pomonella*, Prokopy and Papaj (1988) showed females could distinguish among apple cultivars after experience with them. Because all the cultivars had similar color patterns but quantitatively different emissions of volatile esters, results suggested learning of apple odors. Papaj *et al.* (1989) demonstrated that *C. capitata* females exposed to different fruit types accepted fruit models treated with volatiles from the exposure fruit at higher rates than models treated with volatiles from one of the other fruits. Also, females exposed to fruit generally responded less to models without volatiles compared with naïve females. These results indicate that females learned fruit odor during experience with fruit. However, Prokopy *et al.* (1989) determined that results of experiments testing learning of fruit size and color by female *C. capitata* could be readily explained by size learning, suggesting little or no learning of fruit odors in that case.

Results of this work support our previous findings that Mexican fruit flies do not search instinctively for grapefruits, oranges, or even a native host, yellow chapote. Instead, the data suggest that the flies learn fruit odors after encountering host fruit during general host foraging, then may increase searching efficiency by responding to the learned host odor. Although we used laboratory flies in this work, we believe the results extend to wild flies in nature. As discussed in the Introduction, both laboratory-strain and wild Mexican fruit flies learned about the fruit they experienced and their behavior toward the experienced fruit and similar fruits was similarly modified as a result of the experience (Robacker and Fraser, 2002a; 2002b, 2003). Because behavior of both fly types changes similarly, it is reasonable that both types would learn the same types of cues.

Learning of host odors appears to extend to other herbivorous insects for which instinctive attraction to host odors is known to occur (Landolt, 1989). However, the innate response was not as great as the learned response. For example, attraction of female cabbage looper moths (*Trichoplusia ni*) (Lepidoptera: Noctuidae) (Hubner) to host foliage odors tripled after exposure to the host foliage (Landolt and Molina, 1996). Further, exposure to one host specifically increased attraction to that host over others indicating not only that they can learn host odors but also that they can distinguish among several host odors after experience with one of them. Just how general host odor learning is and what role it plays relative to instinctive attraction to host odors is a question that needs further investigation.

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